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Characterization of the Distribution, Polymorphism, and Stability of Nimodipine in Its Solid Dispersions in Polyethylene Glycol by Micro-Raman Spectroscopy and Powder X-Ray Diffraction

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Abstract

In the present study, a series of solid dispersions of the drug nimodipine using polyethylene glycol as carrier were prepared following the hot-melt method. Micro-Raman spectroscopy in conjunction with X-ray powder diffractometry was used for the characterization of the solid structure, including spatial distribution, physical state, and presence of polymorphs, as well as storage stability of nimodipine in its solid formulations. The effect of storage time on drug stability was investigated by examination of the samples 6 months and 18 months after preparation. Confocal micro-Raman mapping performed on the samples showed that the drug was not uniformly distributed on a microscopic level. The presence of crystals of nimodipine with sizes varying between one and several micrometers was detected, and the crystal size seemed to increase with overall drug content. In samples examined 6 months after preparation it was found that the crystals existed mainly as the racemic compound, whereas after 18 months of storage mainly crystal conglomerates were observed.

Keywords: Solid dispersion, nimodipine, Raman spectroscopy, polymorphism

Introduction

Polymorphism describes different crystal packing arrangements of the same molecular species in its solid state, while the pseudopolymorphs are the solvate and amorphous solid-state forms. Polymorphism investigations are particularly important in drug and product development in the pharmaceutical industry, since certain properties of a formulated product, such as bioavailability and stability, are often directly related to the physicochemical properties of the existing polymorphs in the formulation.[1-3]

X-ray powder diffractometry (XRPD) and solid-state nuclear magnetic resonance are used in the identification of polymorphs, while spectroscopic techniques such as infrared (IR) and Raman can supply information on the short-range structure of polymorphs.[4,5] These techniques can also be used for the quantification of polymorphic composition and degree of crystallinity.[6] The fundamental molecular vibrations are different for each polymorph because of the different packing arrangements, providing a unique "fingerprint" in the form of a spectrum.[7,8] Raman spectroscopy has 3 significant advantages over traditional spectroscopic techniques, such as Fourier transform infrared (FT-IR) spectroscopy. First, it requires no sample preparation. Second, coupling to an optical microscope allows the study of small particles within nonhomogeneous solid sample matrices. Finally, Raman spectra can be obtained noninvasively, even within a sealed transparent container.[9] If the incident light is transmitted through an optical microscope (magnification ×100) before reaching the sample, the light scattered from an area of only a few micrometers can be collected.[7,8,10] The spatial resolution can be further improved in confocal systems, where the collected light passes through a pinhole before reaching the detection system, allowing collection of spectra from a sample volume smaller than 1 μm^3.

Nimodipine is a drug of the 1,4-dihydropyridene type that can be used for the prevention and treatment of ischemic neurological deficits.[11-14] The substance is a racemic mixture of 2 optical antipodes and shows 2 polymorphic forms.[11] Modification I (mod I) is the racemic compound, while modification II (mod II) is the conglomerate. Nimodipine is practically insoluble in water and exhibits low bioavailability after oral administration.[11] Therefore, improving the dissolution characteristics of nimodipine from its oral solid dosage forms becomes a major goal. To date the literature on solid dispersions of nimodipine has been limited.[15-17]

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The development of effective new formulations requires detailed investigation of nimodipine's polymorphism,[11,12,14] particularly since nimodipine dispersed in a solid carrier may exist simultaneously in 2 crystal modifications and as an amorphous material.[17]

Solid dispersions can be used to increase the dissolution rate of a drug with low aqueous solubility, thereby improving its bioavailability.[18-21] One of the most critical aspects in the preparation of such formulations is the ability to assess (and, subsequently, control) the physical state of the drug and its particle size distribution, 2 variables that can significantly influence the drug dissolution rate. Typically, X-ray diffraction (XRD) and scanning electron microscopy (SEM) are used for this task. However, in the case of amorphous drug dispersions, SEM is unable to unequivocally evaluate the drug distribution in the matrix. Confocal micro-Raman spectroscopy, which can provide solid-state characterization of both amorphous and crystalline materials, can be useful in achieving this goal. To the best of our knowledge, the technique has not been used until now in the complete investigation of solid dispersions of drugs, except in 1 study.[22]

The objective of this work is to explore the potential of confocal micro-Raman spectroscopy to qualitatively and quantitatively characterize the solid state of a drug, as well as its dispersion—physical form and spatial distribution—in a polymer matrix (inert drug carrier). The scope of the study is enhanced by including an investigation of the drug's stability (physical state and polymorphism) in the mixtures during long-term storage. The samples examined were solid dispersions of nimodipine in polyethylene glycol (PEG) 4000, prepared according to the hot-melt method. In addition to micro-Raman spectroscopy, XRPD, differential scanning calorimetry (DSC), and hot-stage microscopy (HSM) are used to provide physicochemical characterization of the samples.

Experimental

Materials and Equipment

Micronized nimodipine with an assay content of 101.2%, a melting point of 125°C to 128°C, an aqueous solubility of ~0.5 mg/L, and free solubility in ethanol was supplied by UQUIFA (Barcelona, Spain). The particle size distribution was determined using a Malvern Mastersizer S (633 nm) (Worchestershire, UK) and found to range from ~1 μm (or less) to 29 μm (10% up to 1 μm, 50% between 1 and 9 μm, and 40% between 9 and 29 μm). PEG 4000 with a molecular weight of 3898 g/mol (calculated by OH end groups), a T_m of 54°C (DSC analysis), a moisture content <0.5% (thermogravimetric analysis), and a viscosity of 118 mPa·s (at 20°C, 50% relative humidity [RH]) was obtained from Clariant (Sulzbach, Germany). All other materials and reagents were of analytical grade.

Methods

Preparation of Solid Dispersions

The solid dispersions were prepared by the hot-melt technique. Preliminary HSM tests showed that the temperature for complete dissolution of the nimodipine crystals in the melt of PEG increases with drug content and heating rate, and it was verified that at temperatures between 65°C and 130°C nimodipine starts to dissolve into PEG melt. Thus, physical mixtures of nimodipine and PEG were heated during stirring in a reaction tube immersed in an oil bath to 130°C, under an argon atmosphere. The mixtures were then held to this temperature to ensure that the drug was completely melted and a homogeneous and transparent solution was obtained. Solid dispersions with nimodipine/PEG 10/90 wt/wt, 20/80 wt/wt, 30/70 wt/wt, 40/60 wt/wt, 50/50 wt/wt, and 100/0 wt/wt (control) were prepared. Next, the tubes were immersed in a water bath to quench the melt. The prepared samples were stored at 25°C in desiccator.

To evaluate the effect of long-term storage on the physical structure of nimodipine, the solid dispersions were stored up to 18 months at 25°C and 60% RH.

DSC

DSC studies of the prepared samples were conducted immediately after preparation as well as after storage for 6 months. A Perkin Elmer Pyris 1 DSC (Shelton, CT) equipped with an Intracooler 2P cooling accessory was used. Samples of 5 mg were placed in standard aluminum pans and sealed with a lid. Heating scans of 20°C/min or 100°C/min were applied with a nitrogen purge of 20 mL/min. Fast heating rates are preferred, to prevent changes during scanning.[23]

XRPD

XRPD study of the samples after storage for 10 days as well as after 6 and 18 months was performed over the range 2θ from 5° to 60°, at steps of 0.05°. A Philips PW1710 powder diffractometer (Eindhoven, The Netherlands) with CuKα nickel-filtered radiation was used.

Confocal Micro-Raman Spectroscopy

Raman studies were performed using a HORIBA Jobin Yvon (Edison, NJ) micro-Raman spectrometer (model: LabRAM) equipped with a 632 nm He/Ne laser source, 1800 1/nm grating, and an Olympus BX41 microscope system. Collection of spectra in back-scattering mode was performed at room temperature under the following conditions: ×100 microscope objective, 50 μm pinhole size, 300 μm slit width, and 5 second exposure time. Each spectrum represents the average of 2 measurements. XY scanning (2D

mapping) of the samples was performed at the conditions stated above with the aid of a computer-controlled motorized stage at a step of 0.3 μm in both X- and Y-directions. The effect of surface topography was avoided by automatically focusing the laser beam at each point using the "autofocus" function of the software (LabSpec 4.1 HORIBA Jobin Yvon [Edison, NJ]). The samples were first ground to a fine powder and then spread flat on a glass microscope slide.

HSM

A polarizing optical microscope (Nikon, Optiphot-2, Tokyo, Japan) equipped with a Linkam THMS 600 heating stage, a Linkam TP 91 control unit, and a Jenoptic ProgRes C10plus camera with Capture Pro 2.1 software was used for HSM observations.

Results and Discussion

Characterization of the Modifications of Nimodipine

Nimodipine is a substance that can be obtained in either the amorphous or the crystalline state depending on the treatment and storage conditions. Two different crystal modifications of the crystalline substance are found.[11] Commercial nimodipine samples are in fact racemic mixtures of the 2 optical antipodes.[11,13] An interesting subject of chemical research has been the relationship between the racemic and chiral crystals of 2 opposite and resolvable enantiomers (*d* and *l*). Three crystal forms may appear for substances showing optical antipodes: the racemate or racemic compound, a crystal containing both *d* and *l* in the same unit cell; the enantiomorph, a chiral crystal of *d* or *l*; and the conglomerate, an equimolar mixture of the *d* and *l* enantiomorphs. Crystal mod I of nimodipine refers to the racemic compound, while mod II refers to the conglomerate.[11] Mod II is favored by crystallization at room temperature, as well as by crystallization in the presence of isopropanol or ethanol traces. If the homogeneous mixture of the optical antipodes cocrystallizes, the racemic compound is formed. If the antipodes are locally separated, crystallization results in a mixture of the crystals of the pure antipodes (conglomerate). Below 88°C the less soluble mod II is the most stable. The system is enantiotropic and crystals of mod I transform to mod II at room temperature.[11] To achieve dissolution rates that are as high as possible, the drug must be kept in the amorphous state or, if it partly crystallizes in the formulations, then its transformation from mod I to mod II crystals must be prevented.

Figure 1 presents a series of wide-angle XRPD patterns for various nimodipine samples. The pattern of the original ("as received") drug sample used in this study corresponds to that of nimodipine crystal mod I. The one obtained after crystallization from solution in ethanol corresponds to the nimodipine crystal mod II. The XRPD patterns for the 2 crystal modifications are very similar, except for the crystalline reflections at low angles 2θ: for example, characteristic peaks at 2θ = 6.7° for mod I, and at 2θ = 10.40° for mod II can be easily observed. Furthermore, the pattern of a melt-quenched nimodipine sample even after storage for 10 days at room temperature shows very low crystallinity. This finding indicated that nimodipine can be obtained in an essentially amorphous state from a melt-quenching procedure. The DSC study showed a glass transition temperature T_g = 20°C for the drug. It was found that the substance, in contrast to many other drugs, does not show crystallization during cooling from the melt, or during heating an amorphous sample from room temperature up to the melting point (126°C), at least when the cooling or the heating rate is faster than 5°C/min. The melt-quenched sample crystallized during long-term storage for 6 months, and its pattern appears in Figure 1, along with the pattern for after 18 months. The reflections were basically those of crystal mod II.

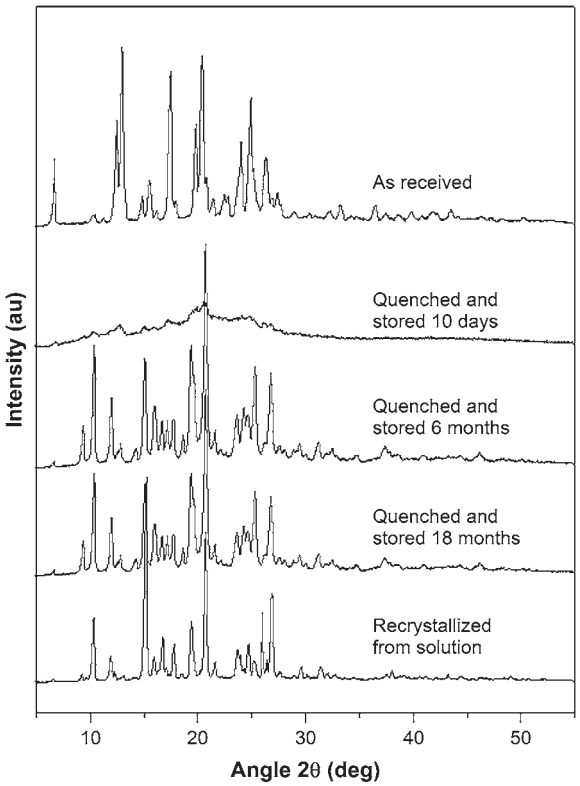

Figure 1. X-ray powder diffractometry patterns of various nimodipine samples.

Confocal micro-Raman spectroscopy was used to study the polymorphism of nimodipine. XRPD relies on the supramolecular (physical) structure—that is, the extent of 3-dimensional order—to distinguish between the crystalline and the amorphous state of the drug and the specific characteristics of the crystalline structure. Despite the fact that the crystal

modifications are the result of the arrangement of the antipodes, XRPD cannot directly detect differences on a molecular level. Micro-Raman spectroscopy, however, allows examination of the intermolecular (chemical) structure of a substance and therefore enables the characterization of not only the crystalline drug but also the amorphous drug.

The 2 crystal modifications of nimodipine can be distinguished by means of vibrational spectroscopy, as they exhibit different (Raman and IR) spectra, meaning different intensities at the characteristic peaks of the drug.[11,17] As can be seen in Figure 2, the peak intensity at 1642 cm^{-1} (C=C bond stretch of the dihydropyridine ring) for the racemic crystal (mod I) is significantly lower than that at 1347 cm^{-1} (symmetric stretching vibrations of the $–NO_2$ group[24]). As a result, this intensity ratio for mod I is much larger than 1. In contrast, the same ratio for the homochiral crystal (mod II) gives a value much smaller than unity. Interestingly, the intensities of the 2 peaks are almost balanced in the spectrum of the amorphous drug, resulting in a $I_{1347} \div I_{1642}$ ratio close to unity. The values of the intensity ratios corresponding to each physical state of the pure drug are shown in Table 1. The tabulated mean values are for 100 measurements taken from each sample. This significant variation in the $I_{1347} \div I_{1642}$ ratio between the amorphous and 2 crystalline states of the drug may be useful in determining the physical state of nimodipine in its solid dispersions.

Characterization of Solid Dispersions

In his review article, Craig underlined 4 key problem areas in the formulation and use of solid dispersions: the solid structure, the mechanism by which dissolution enhancement occurs, the stability of the dispersions during storage, and the poor understanding of the in vitro/in vivo correlation.[20] The first 3 areas are related to the physical characteristics of the dispersions and, consequently, to the manufacturing process employed for their preparation. As mentioned in the introduction, the objective of this study was to explore the capabilities of micro-Raman spectroscopy in the characterization of the solid state of nimodipine in a series of solid

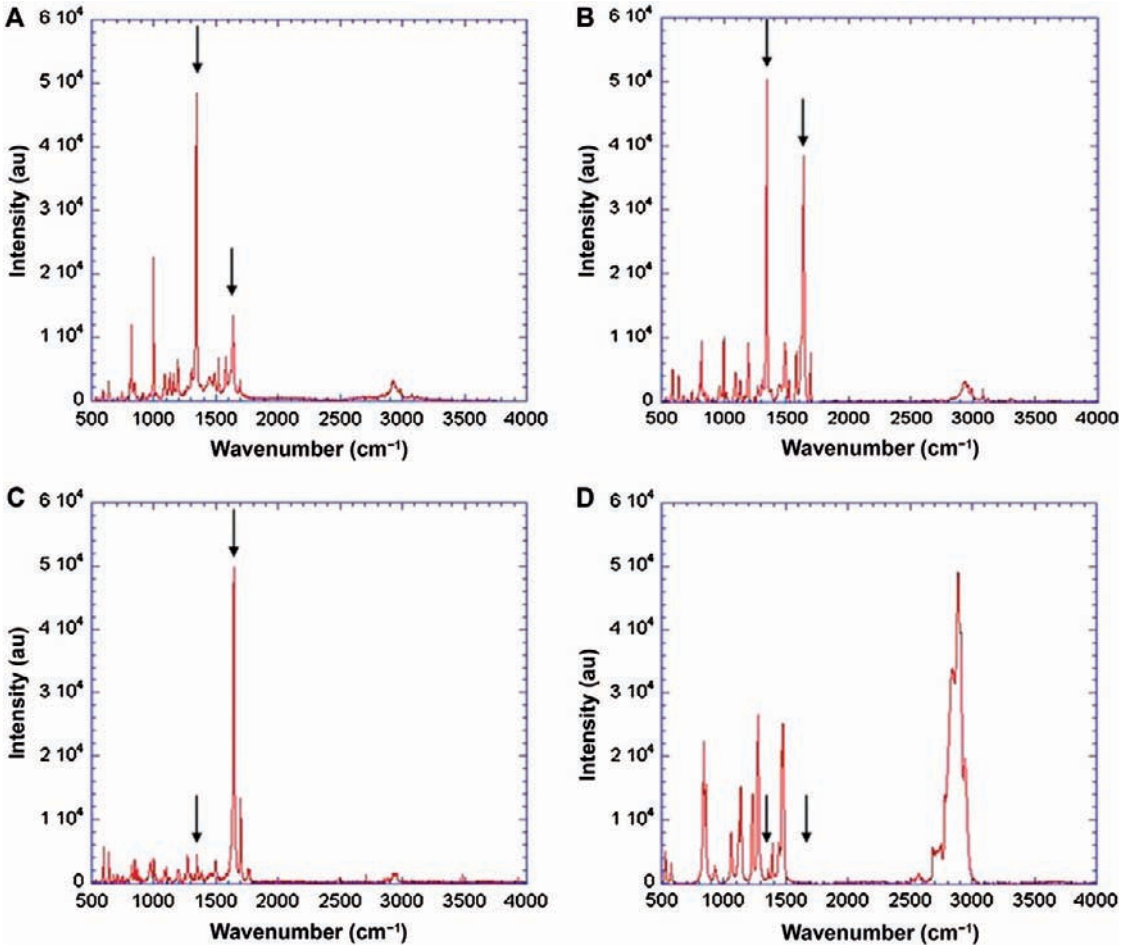

Figure 2. Raman spectra of nimodipine existing in different forms: (A) crystal of modification I; (B) amorphous phase; (C) crystal of modification II; (D) Raman spectrum of pure polyethylene glycol. For (A), (B), and (C), the polymorphs can be distinguished on the basis of the peak intensities at 1347 and 1642 cm^{-1}, as indicated by the arrows. In (D), the arrows indicate that no peaks exist at the above wavenumbers.

Table 1. Intensity Ratios Corresponding to the 3 Types of Nimodipine Examined*

Phase	Structure	Peak 1 (cm^{-1})	Peak 2 (cm^{-1})	Intensity Ratio, R (=$I_1 \div I_2$)	
				Mean	SD
Modification I	Crystalline	1347-1348	1642-1643	3.67	0.58
Amorphous	Amorphous	1349-1350	1646-1648	1.22	0.17
Modification II	Crystalline	1348-1349	1643-1644	0.171	0.035

*The columns labeled Peak 1 and Peak 2 indicate the wavenumber range of 2 characteristic band assignments.

dispersions in PEG prepared by the hot-melt technique. Since nimodipine has no detectable elements such as Cl, F, Br, or Na, it is not possible for its particles to be distinguished with SEM–energy dispersive spectroscopy or transmission electron microscopy, which our team has used in previous studies.[25-27] Additionally, SEM can detect only well-formed crystal particles and is unable to give information on particle distribution in cases where the dispersed drug is in the amorphous state. On the other hand, powder XRD is used to study collectively the bulk of a sample, since it detects crystalline drug over a flat sample of significant surface area (~3 × 3 cm^2), giving an averaged picture for crystallinity and crystal modifications; however, it cannot detect, or provide information on, the amorphous phase. For this reason micro-Raman spectroscopy was selected as an alternative technique that can possibly provide a more complete sample characterization by combining the capabilities of the previous 2 methods. By surface scanning an area, confocal micro-Raman spectroscopy can offer selective image contrast of each molecular component of a mixture on a plane, while optical microscopy provides only a surface mapping of the whole mixture, where the contrast appears usually in terms of reflective index or reflectivity. The principle of Raman imaging consists of scanning the surface at a fixed wavenumber characteristic of a component of the mixture (eg, stretching vibrations of the NO_2– group of nimodipine). Each time the incident monochromatic light meets the sample, the intensity of a peak at a Raman shift characteristic of the component of interest is collected and measured, while those of the other components are omitted.[8]

Since the FT-Raman and micro-Raman techniques have begun to be used in the study of pharmaceutics only recently, a limited number of studies currently exist in the literature.[28-34] Before now, mapping has been used in only 1 study of solid dispersion systems.[22]

The preparation and storage conditions of the solid dispersions play a critical role, as changes may alter the dissolution characteristics of the active ingredients over time.[35-41] Drugs dispersed in the amorphous phase are expected to be more soluble than the crystalline or polymorphic forms. However, amorphous drugs are less stable and more sensitive to physical alterations during storage. Thus, examination of the morphology of the drug and the polymer matrix in the solid dispersions is very important to test the possible effects of storage. Methods for monitoring the stability of the amorphous state of the drug dispersed in the polymer have been explored for a long time. DSC, differential thermoanalysis, solution calorimetry, IR spectroscopy, XRPD, and only recently FT-Raman spectroscopy have been used.[42,43] XRPD and micro-Raman spectroscopy are used comparatively in the present study.

Identification of the crystal modifications of nimodipine in its mixtures with PEG was attempted by using as criterion the intensity ratio of the Raman bands at 1347 cm^{-1} and 1642 cm^{-1} ($I_{1347} \div I_{1642}$). In light of the fact that the value of this ratio was so distinctly different among the physical states of the drug in its pure form, we examined whether it could also serve as a tool for the quick characterization of nimodipine in its solid dispersions. Figures 3A and 3B show examples of (XY) micro-Raman scans performed on samples with nimodipine/PEG weight ratios 10/90 and 50/50 wt/wt, respectively, 6 months after their preparation. Figures 3C and 3D show scanning results from samples of the same compositions (90/10 and 50/50, respectively) after 18 months of storage. The color bar in the images indicates the value of the intensity ratio $I_{1347} \div I_{1642}$. In the scans obtained from samples after 6 months of storage, areas of various size that displayed a very high intensity ratio (yellow/white, $I_{1347} \div I_{1642} \geq 3.5$) were seen to be surrounded by continuous areas with values in the range $0.25 < I_{1347} \div I_{1642} < 3.5$. As can be seen in Figure 3A, dark blue areas ($I_{1347} \div I_{1642} \leq 0.25$) were found to coexist with areas displaying higher intensity ratios. The scans obtained from samples stored for 18 months had a totally different appearance (Figures 3C and 3D). Here, the yellow areas had almost disappeared, while dark blue areas were more frequent and larger than before. The above observations were consistent among all the micro-Raman scans (5 for each concentration).

At first glance, a challenge in interpreting these scans arises from the big disparity of the intensity ratio values over the whole sample, especially in the large areas represented by intermediate colors ($0.25 < I_{1347} \div I_{1642} < 3.5$) that cannot be

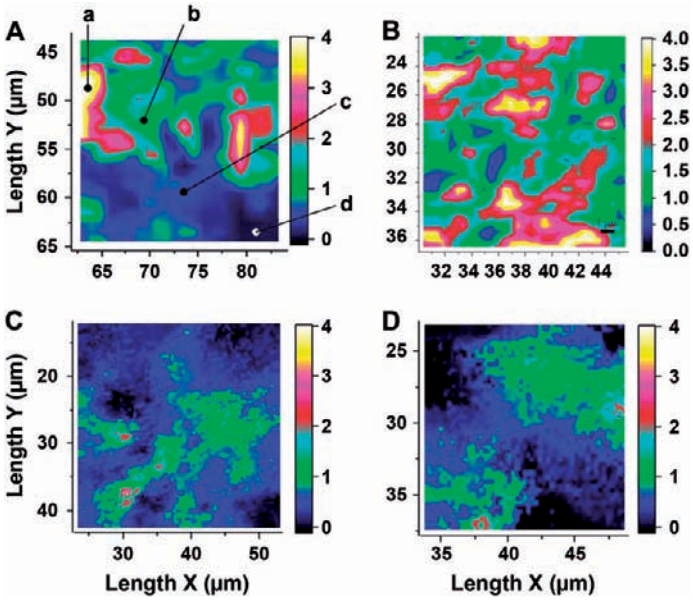

Figure 3. Micro-Raman XY scans of samples prepared by the melt method. Scans (A) and (B) correspond to nimodipine/PEG 10/90 and 50/50 wt/wt, respectively, and were obtained 6 months after sample preparation. Scans (C) and (D) correspond to nimodipine/PEG 10/90 and 50/50 wt/wt, respectively, and were obtained 18 months after sample preparation. The color map indicates spatial variation of the intensity ratio $I_{1347} \div I_{1642}$. PEG indicates polyethylene glycol. Points a, b, c, and d in Figure 3A correspond to spectra a, b, c, and d in Figure 4E-H.

readily classified as corresponding to completely crystalline or amorphous nimodipine. To investigate the physical state of nimodipine in its solid dispersions with PEG and whether a meaningful relationship exists between this and the value of the $I_{1347} \div I_{1642}$ intensity ratio, a close examination of the Raman spectra corresponding to regions of different color in the scans was performed. Identification of the physical state of nimodipine in the mixture was attempted by comparing the spectra obtained from mixtures with those of pure nimodipine, as seen in Figure 4. The first row (Figures 4A, 4B, 4C, and 4D) depicts the Raman signatures of pure nimodipine (and PEG) in selected spectral windows where differences between the racemic and homochiral crystals of the drug can be observed. Figure 4A shows that mod I exhibits a single band at 1198 cm^{-1} (corresponding to the stretching vibration of the C-C bond between the aromatic and pyridine rings[24]), whereas in the spectrum of mod II a split of this band had occurred. The amorphous material showed a distinct band at 1197 cm^{-1} and a poorly resolved peak at slightly higher wavenumbers. Figure 4B shows the peaks assigned to the symmetric stretching vibrations of the $–NO_2$ group. This peak exhibited high intensity at 1347 cm^{-1} in the spectra of mod I. In the mod II spectrum, the peak was significantly weaker and slightly shifted to higher wavenumbers (1348-1349 cm^{-1}). The amorphous material displayed a broad band centered at ~1348 cm^{-1}. The relation between the bands of mod I (1642 cm^{-1}) and mod II (1644 cm^{-1}) was the opposite in Figure 4C (C=C stretch bond), where the latter exhibited a band of significantly higher intensity. The band for the amorphous material was broad and centered at 1647 cm^{-1}, and was of higher intensity than that of mod II. Finally, in Figure 4D the bands corresponding to the stretching vibrations of the >C=O groups of the molecule are shown. The bands in this part of the spectrum were typically weak, although that of mod II (1699 cm^{-1}) was generally significantly larger than the others (1700 cm^{-1}). An additional, less intense, band (1692 cm^{-1}) could be observed in the spectrum of mod I.

The spectra of pure nimodipine described above were subsequently used as reference for identifying the physical state of the drug in its solid dispersions with PEG. The examination involved over 200 spectra collected from various points in Raman scans obtained from mixtures with concentrations 10 to 50 wt% in nimodipine. An example is given in Figures 4E-4H, which show the corresponding spectra of the points marked as A through D in Figure 3A. As can be seen, the Raman signatures (band shapes and locations) of points A ($I_{1347} \div I_{1642} \geq 3.5$) and D ($I_{1347} \div I_{1642} \leq 0.25$) were very similar to those of mod I and mod II, respectively. This finding was consistent (and exclusive) for all points collected from areas with intensity ratios outside the range 0.25 to 3.5. Specifically, the examination of 20 samples from areas with a ratio greater than 3.5 (yellow-colored areas) gave an average value of 3.84 (SD ±3.67; median 3.69). The examination of 20 samples from areas with a ratio less than 0.25 (dark blue areas) gave an average value of 0.18 (SD ±0.05; median: 0.19). On the basis of this result it can be concluded that the ratio $I_{1347} \div I_{1642}$ can be used as a criterion for identifying the presence of crystals and their modification in the PEG/nimodipine solid dispersions. The Raman signatures of points from regions with a ratio within the range $0.25 < I_{1347} \div I_{1642} < 3.5$ were also examined. The Raman spectra collected from these regions did not fully conform to any of the spectra observed for the pure crystalline or amorphous nimodipine. Points B (green area) and C (light blue) were 2 examples of that case. From Figures 4E to 4H it can be seen that the spectrum of point B had a lot of similarities with that of mod I, but the shape and location of its band at 1700 cm^{-1} resembled that of the amorphous material. Raman spectra collected from samples of 1 wt% nimodipine in PEG (results not shown) gave the same peak assignments and $I_{1347} \div I_{1642}$ ratios as those obtained from amorphous pure nimodipine. It can therefore be inferred that both forms of nimodipine (amorphous and mod I) might be present in this location. On the other hand, point C followed loosely the characteristic bands of mod II, suggesting that some of the drug in this area was present in the form of crystals of mod II. From Figure 4E it can be seen that the spectra of these 2 points also contained bands that belonged to the molecules

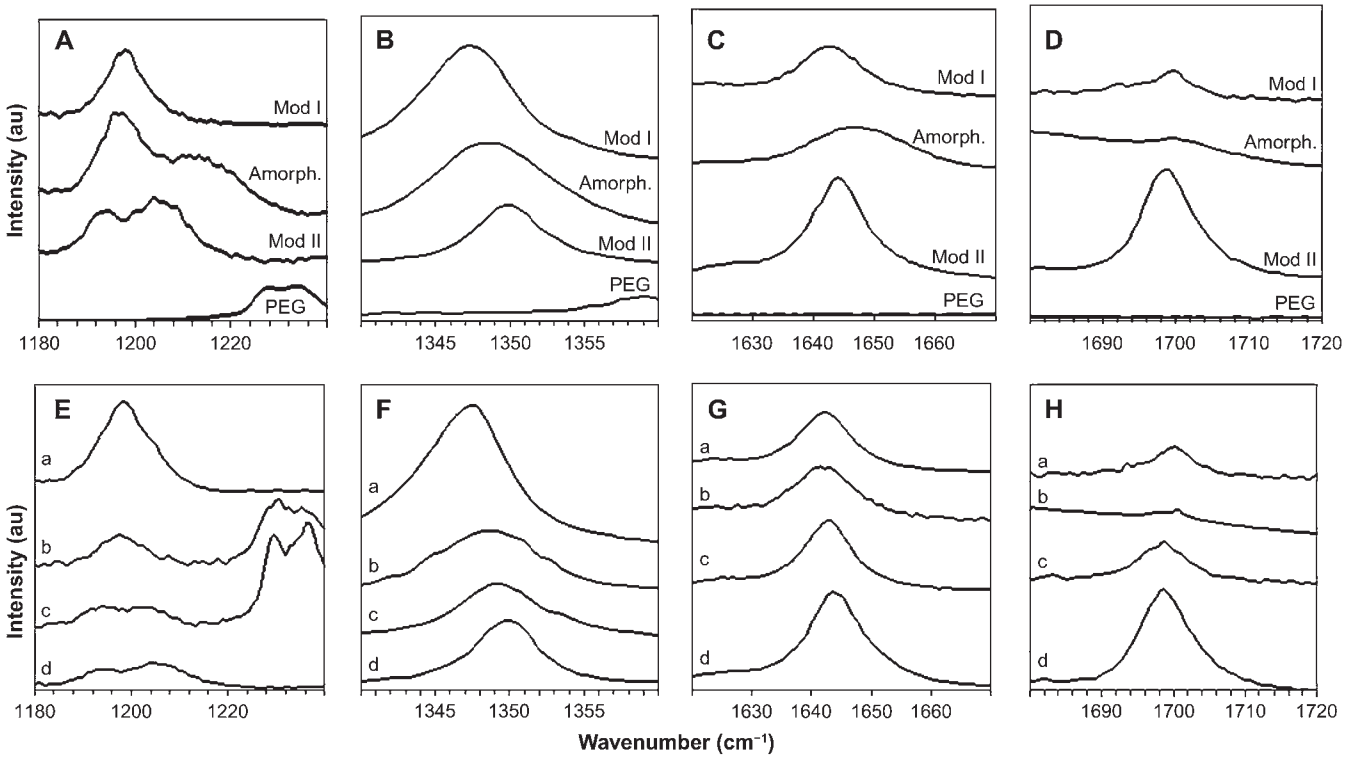

Figure 4. (A), (B), (C), and (D) Details of Raman spectra obtained from samples of pure nimodipine; (E), (F), (G), and (H) nimodipine/PEG solid dispersions. Mod indicates modification; Amorph, amorphous; PEG, polyethylene glycol. Spectra a, b, c, and d in Figure 4E-H correspond to points a, b, c, and d in Figure 3A.

of PEG, an indication that the drug did not exist in pure form. It should also be mentioned that, for both points, the characteristic peaks of their spectra were found to be less intense than those corresponding to the crystalline modifications and also slightly shifted to different frequencies. Intensity attenuations and band shifts result from local variations in drug structure and composition and indicated that the intermolecular interactions of the nimodipine with its surroundings were not the same everywhere. In fact, shifts in the bands of nimodipine (occasionally up to 4 cm^{-1}) were frequently observed to occur in spectra collected from different locations in the scans. Notably, in some of the spectra (approximately 30 out of 200) shifts in the band assigned to NO_2-stretch (normally appearing at 1642-1644 cm^{-1}) were also found, resulting in erroneous calculations of the $I_{1347} \div I_{1642}$ ratio. Taking into account all these discrepancies, it was concluded that the values of the ratio $I_{1347} \div I_{1642}$ in the range of 0.25 to 3.5 indicated material existing in a heterogeneous state, but this parameter could not be used alone for an in-depth interpretation of the physical state of the drug in the mixtures.

In summary, we showed that examination of spectra corresponding to $0.25 < I_{1347} \div I_{1642} < 3.5$ revealed that nimodipine in these regions coexisted with PEG and may be present simultaneously in more than 1 form. The composition of these regions, which was found to vary with location, can be best described as heterogeneous. Identification of domains within these areas where nimodipine existed in only a single form could not be performed, as the size of these domains was below the resolution capabilities of the instrument used in this study. Therefore, we can only speculate that these areas contained crystals of nimodipine of molecular to submicron-sized dimensions and/or amorphous regions formed in the presence of PEG. Detailed analysis of the Raman spectra, for instance, through peak deconvolution and subsequent integration of the area under each Raman peak, can possibly provide more information on the composition and amounts of nimodipine present in each domain, but this analysis often proves laborious.

The conclusions drawn from this analysis can now be used toward an interpretation of the micro-Raman maps of Figure 3. Having established that the ratio $I_{1347} \div I_{1642}$ may be used for the identification of crystalline domains of the drug in the PEG/nimodipine melts, white/yellow domains ($I_{1347} \div I_{1642} \geq 3.5$) in the scans can be taken to represent crystals of mod I, while dark blue domains ($I_{1347} \div I_{1642} \leq 0.25$) can be taken to represent crystals of mod II. In the scans corresponding to samples examined 6 months after preparation, crystals with sizes from one to several micrometers could be seen. Overall, it appeared that the crystals (mod I or mod II) of nimodipine seemed to coexist in highly distributed and discontinuous microdomains throughout the sample volume. Several scans (5 per concentration) performed on

these samples indicated a trend of increasing crystal size with overall drug content. This semiqualitative observation was also supported by HSM studies (not shown) performed above 60°C so that the melting of the polymer matrix (PEG 4000) would allow observation of the size of the drug crystals. These observations could be rationalized in terms of an increasing degree of crystallinity as a result of supersaturation observed with increasing drug content. An estimate, obtained through XRPD data analysis, showed that the drug's crystallinity was 34, 43, 55, 64, and 67 wt% in solid dispersion containing 10, 20, 30, 40, and 50 wt% nimodipine, respectively. The crystallinity was assessed by using as standards physical mixtures of nimodipine/PEG containing the drug in its "as received" form (pure crystalline nimodipine). The results were obtained by first subtracting from the XRPD patterns the peaks corresponding to crystal reflections of PEG and then integrating the area under the peaks of crystalline nimodipine.

Although crystals could be seen in the micro-Raman scans, the majority of the mapped areas consisted of heterogeneous domains ($0.25 < I_{1347} \div I_{1642} < 3.5$) representing mixtures of nimodipine with PEG. As mentioned earlier, Raman spectra collected from these domains provide indications that the drug existed in different physical states, the length scales of which (molecular to submicron) are too small to be resolved with the present technique.

Samples with drug concentration 10 and 50 wt% were also examined after 18 months of storage (Figures 3C and 3D, respectively). The appearance of these samples was in stark contrast to that of the samples after storage for 6 months. Specifically, areas where the drug existed in crystalline mod II seemed to have increased, while crystals of mod I had almost disappeared. As discussed previously, the scans showed that the majority of the sample is occupied by heterogeneous domains.

Examination of the drug stability with time was also performed by means of XRPD. The patterns of the solid dispersions were recorded at 3 different times: 10 days, 6 months, and 18 months after preparation of the samples. The study showed that no substantial change occurred in the sample over the period between the first 2 measurements. However, the XRPD traces recorded at 18 months were quite different compared with the initial measurements, possibly because of crystal modification of the drug. The patterns after 10 days of storage showed peaks of nimodipine crystal mod I, while the peaks in the patterns after 18 months were in principle of mod II, though there were some residual peaks corresponding to mod I. For pure nimodipine, crystallization below 88°C favors appearance of mod II. Our tests in the case of pure melt-quenched nimodipine showed that in the XRPD pattern after 18 months of storage at 25°C only peaks for mod II crystals appeared. Although the drug appeared to be effectively stabilized in the dispersion in the polymer for times up to 6 months, the storage period of 18 months was long enough for changes in the crystalline structure of the drug to occur. In general, the XRPD results were in qualitative agreement with those obtained from Raman mapping in terms of the changes in the crystalline modification of the drug with time.

Conclusions

In the present study, characterization of the distribution and physical state of the drug nimodipine in its solid dispersions with PEG was attempted by means of confocal micro-Raman spectroscopy and powder XRD. Two-dimensional Raman maps displaying the spatial variation of the intensity ratio of 2 characteristic peaks of the Raman spectrum of nimodipine ($I_{1347} \div I_{1642}$) were used for the examination of samples with overall nimodipine content between 10 and 50 wt%. The mapping results revealed that part of the drug existed in the form of crystals with sizes from one to several micrometers. The majority of the mapped areas, however, were found to constitute heterogeneous regions where nimodipine coexisted with PEG. Raman spectra obtained from points within these regions were not consistent and seemed to vary randomly with location, which suggested that nimodipine was not uniformly distributed and might, locally, be present simultaneously in more than 1 form. Further information on the distribution and physical state of nimodipine inside these heterogeneous regions may be possibly obtained through detailed analysis of the collected spectra; this laborious task will be the objective of future research.

Micro-Raman spectroscopy and XRPD were also used to study the effect of storage time on the stability of the drug, and the observed results were in qualitative agreement. Specifically, in samples examined 6 months after preparation it was found that the drug crystals were mainly heterochiral (mod I). Examination of the same samples after 18 months of storage showed the presence of only homochiral crystals (mod II), indicating that this storage period was long enough for the transformation in the crystalline structure of the drug to occur.

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